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Animal Studies

Proliferation of pneumocyte II cells in prolonged exposure to 1% CO₂

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Douglas, W. H. J., K. E. Schaefer, A. A. Messier, and S. M. Pasquale. 1979. Proliferation of pneumocyte II cells in prolonged exposure to 1% CO₂. Undersea Biomed. Res. Sub. Suppl.: S135-S142. —Guinea pigs were exposed to 1% CO₂ in a mixture of 21% O₂, balance N₂ and were killed at weekly intervals, together with control animals housed in the same type of environmental chamber and exposed to normal ambient CO₂. The exposed animals showed a persistent elevation of PaCO₂, averaging about 4 mmHg, and a small decrease in pH (-0.04 units). During the whole exposure period standard bicarbonate remained 1-1.5 mEq below control levels, indicating a failure of the kidney to increase bicarbonate reabsorption. Electron microscopic studies after 4 and 6 weeks of exposure to 1% CO₂ showed ultrastructural changes of the lungs, consisting of marked increases in the size and number of pneumocyte II cells that were still present two weeks and to a lesser extent four weeks after recovery. Changes in the pneumocyte II cell were postulated to be compensatory reactions to impairing CO₂ effects on the alveolar lining cell (Type I cell).

CO₂ toxicity
ultrastructure, lungs
acidosis

Exposure to concentrations of 3% and higher CO₂ levels produced pulmonary hyaline membranes in guinea pigs (Niemoeller and Schaefer 1962; Schaefer, Avery, and Bensch 1964; Bensch, Avery, and Schaefer 1964). The incidence of hyaline membrane formation decreased progressively during chronic CO₂ exposure after compensation of the respiratory acidosis was reached, indicating a non-specific acidosis effect (Schaefer et al. 1964). Ultrastructural changes associated with the CO₂-induced transient hyaline membrane formation consisted of a disappearance in osmiophilic lamellar bodies (OLB's) of the type II pneumocyte. A decrease in surfactant production, indicated in the rise of minimal surface tension, was associated with these morphological changes (Schaefer et al. 1964).

Since previous studies have shown that the ultrastructural changes of the lungs are related to an unspecific acidosis effect, the question arose as to whether or not prolonged exposure to 1% CO₂, which produces a long-lasting slight acidosis without renal compensation, would also lead to ultrastructural changes of the lung.

MATERIALS AND METHODS

Twenty-nine caesarean-section-born guinea pigs of the Hartley strain, maintained free of respiratory disease and weighing between 400–600 g, were exposed to 1% CO₂ in a 21% O₂, balance N₂ mixture for periods up to 6 weeks in environmental chambers with temperature and humidity control (Sherer-Gillette). Another group of 12 animals was exposed for four weeks and allowed to recover for 4 and 8 weeks, respectively. The environmental temperature was kept at $25.6 \pm 1.1^\circ\text{C}$ and the humidity between 65 and 75%. The gas mixtures were prepared by mixing proportional amounts of CO₂ to air; oxygen was added from a high-pressure cylinder. The air within the chamber was recirculated 12 times a minute. With this fast and large turnover of chamber air, mixing of CO₂ and air was nearly instantaneous. The carbon dioxide concentration in the chamber was continuously monitored with a Beckman infrared CO₂ analyzer and the oxygen content was sampled intermittently with a Beckman O₂ analyzer. The CO₂ concentrations were kept at $1\% \pm 0.1\%$ and the oxygen concentration at $21\% \pm 0.5\%$. Ammonia vapor was absorbed by boric acid placed in the chamber. The chamber was opened each morning for a period of 3–5 min to fill the water and food containers and to remove urine and feces.

Thirty-six litter mates of the first group of animals were kept in a second environmental control chamber under exactly the same conditions, except that they were not exposed to CO₂. As each experimental group of 6 animals was killed, a control group of 3–5 animals was also killed to obtain control data on lungs, bones, and kidneys; bones and kidneys were used in separate studies. For blood gas studies, 8 control animals were used with each experimental group to obtain a statistically significant number of satisfactory arterial blood samples (those in which the oxygen partial pressure was above 50 mmHg, a criterion set in our laboratory for acceptable arterial blood samples). Particular emphasis was given to the collection of proper blood gas data since they served as a basis for different studies of the effects of 1% CO₂ carried out in association with the blood gas studies.

Before blood sampling, the animals received 40 mg pentobarbital/kg body wt subcutaneously and were then returned to the CO₂ exposure chamber. The anesthesia was usually effective after approximately 5 min, at which time the animals were taken out of the exposure chamber and immediately placed under a mask through which they breathed a CO₂ gas mixture identical to the one to which they had been exposed. Blood samples were drawn from the abdominal aorta. Blood pH, PCO₂, and PO₂ were determined with an Instrumentation Laboratory blood gas and pH analyzing system and standard bicarbonate was also determined.

Electron microscopic studies of lung ultrastructure

Minced lung from both control and experimentally treated animals was prepared for electron microscopy by fixation in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 1 h and postfixed for 30 min in 1% osmium tetroxide in a graded series of acetone in water and embedded in Epon-Araldite®. Before sectioning, the samples were coded to prevent ready identification of tissue. Ultra-thin sections were cut for electron microscopy with a diamond knife in a Porter-Blum MT-2 ultramicrotome, stained in magnesium uranyl acetate and lead

TABLE 1
EFFECT OF PROLONGED EXPOSURE TO 1% CO₂ ON WEIGHT AND ACID BASE IN GUINEA PIGS

Length of Exposure	Experimental						Control					
	Weight, g	PCO ₂ , mmHg	PO ₂ , mmHg	pH units	Bicarbonate, mM/liter	Standard	Weight, g	PCO ₂ , mmHg	PO ₂ , mmHg	pH units	Bicarbonate, mM/liter	Standard
1 Week	Mean 521	37.6	64.6	7.381*	20.6		537	34.0	65.2	7.429	21.5	
	SE 7	1.3	12.5	0.009	0.4		8	1.6	4.4	0.016	0.7	
	n (8)	(8)	(8)	(8)	(8)		(8)	(8)	(8)	(8)	(8)	
2 Weeks	Mean 560	37.2	55.3	7.342*	20.6		568	36.4	66.1	7.405	22.3	
	SE 4	2.4	10.0	0.014	0.4		5	1.2	3.0	0.008	0.5	
	n (5)	(5)	(5)	(5)	(5)		(7)	(7)	(7)	(7)	(7)	
3 Weeks	Mean 577	38.9*	56.6	7.380	19.5*		584	33.6	71.1	7.422	21.7	
	SE 6	0.4	12.0	0.013	0.5		3	0.8	4.3	0.019	0.5	
	n (5)	(5)	(5)	(5)	(5)		(7)	(7)	(7)	(7)	(7)	
4 Weeks	Mean 607	40.9*	58.3	7.358*	20.3		602	34.0	61.3	7.406	20.9	
	SE 5	1.7	8.3	0.003	0.3		5	1.5	3.8	0.017	0.4	
	n (6)	(6)	(6)	(6)	(6)		(7)	(7)	(7)	(7)	(7)	
6 Weeks	Mean 655	36.1	71.3	7.405	21.1		662	34.2	62.2	7.424	21.9	
	SE 7	1.0	3.5	0.004	0.4		4	0.9	4.6	0.012	0.3	
	n (5)	(5)	(5)	(5)	(5)		(7)	(7)	(7)	(7)	(7)	
Recovery After 4 Weeks' Exposure												
4 Weeks on 1% CO ₂ + 2 weeks on air	Mean 790	34.0	67.5	7.371	20.0							
	SE 10	2.0	4.0	0.029	1.1							
	n (6)	(6)	(6)	(6)	(6)							
4 Weeks on 1% CO ₂ + 4 weeks on air	Mean 899	35.9	55.4	7.367	20.2							
	SE 9	1.4	8.7	0.007	0.5							
	n (6)	(6)	(6)	(6)	(6)							

*Significantly different from controls at the 5% level or better.

citrate, and examined and photographed in a Philips EM-300 electron microscope. These electron micrographs were examined and evaluated by judges who did not know which samples were from experimental or control animals. The ultrastructural changes in pulmonary tissue induced by 1% CO₂ caused alterations in type II cells that were readily identified in coded samples. Ten lung specimens were prepared for each animal. For each of the specimens, 15 photographs were taken (150 photographs per animal). The methodology used can only indicate qualitative change or at best a semi-quantitative change in the experimentally treated tissue.

Further details on the electron microscopic methods used have been reported elsewhere (Redding et al. 1975; Douglas and Teel 1976). All statistical comparisons of physiological and morphological data were done by Student's *t*-test.

RESULTS

Data on body weight, arterial PCO₂, PO₂, pH, and standard bicarbonate of guinea pigs exposed to 1% CO₂ and control animals are presented in Table 1. Body weight increased in the same way in both groups of animals. There was no discernible effect of exposure to CO₂. Exposure of guinea pigs to 1% CO₂ for six weeks caused a persistent elevation of PaCO₂, averaging about 4 mmHg, resulting in an acidosis (indicated by a small but statistically significant decrease in pH at 1, 2, and 4 weeks). Average PaO₂ values ranged between 55 and 71 mmHg. Standard bicarbonate, which gives a measure of the average kidney bicarbonate reabsorption, remained an average of 1.5 mEq below the control level.

In electron microscopic studies of the lungs, the following cell types were examined and compared in control lungs and lungs of animals exposed to 1% CO₂: capillary endothelial cells and their basement membranes; type I cells of the alveolar epithelium; type II cells of the alveolar epithelium; fibroblast in the interstitial connective tissues; alveolar macrophages; ciliated epithelial cells of terminal bronchioles; and clara cells of the terminal bronchioles. Exposure to 1% CO₂ for 7, 14, and 21 days did not alter the fine structural appearance of any of these cellular components compared to those of control lungs.

After exposure of the animals to 1% CO₂ for 28 days, there were changes in cell fine structure, particularly in the type II alveolar pneumocytes (granular pneumocytes). There was a hypertrophic increase in the size of the CO₂-exposed type II cells compared to control cells. In addition, the size and number of osmiophilic lamellar bodies were increased in type II cells in the CO₂-stressed lungs compared to control lungs. Another observation was that in lungs exposed to CO₂, groups of 2-4 type II cells were often observed. Clusters of type II cells were never observed in lung sections from control animals. These changes (increase in size of type II cells, increase in size and number of osmiophilic lamellar bodies in type II cells, and increase in numbers of type II cells) suggest that after 28 days' exposure to 1% CO₂, guinea pig lung responds to CO₂ stress by increasing type II cell activity. The same ultrastructural changes were observed after six weeks of exposure to 1% CO₂ and continued to persist after two and four weeks' recovery on air following four weeks of exposure to 1% CO₂.

Figure 1 shows representative pneumocytes from guinea pigs exposed to 1% CO₂ for four weeks. The type II cells of the exposed animals were clustered together and the osmiophilic lamellar bodies (OLB's) were enlarged in size. Moreover, there were more osmiophilic lamellar bodies in these cells than in control cells.

Table 2 shows data on cell diameter, number of lamellar bodies, and lamellar body diameters of type II pneumocytes in guinea pigs exposed to 1% CO₂ compared with those from control animals. For the first three weeks of exposure to 1% CO₂, no alterations were ob-

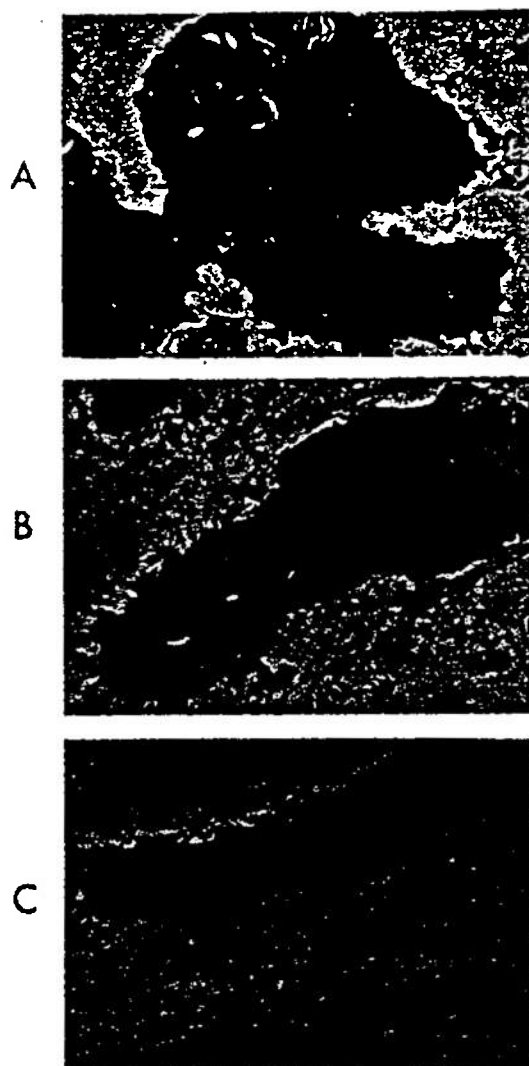


Fig. 1 Representative type II pneumocytes from guinea pigs exposed for 4 weeks to 1% CO₂. A, 3 type II cells clustered together; osmiophilic lamellar bodies (OLB's) are present in all 3 cells and are enlarged in size; B, one hypertrophic type II cell. There are more osmiophilic lamellar bodies in this cell than in controls; C, OLB's are increased in size; line marker on each electron micrograph denotes 2 μm.

served. After 4 and 6 weeks of exposure to 1% CO₂, however, significant differences were found in cell diameter, number of lamellar bodies, and diameter of lamellar bodies. These quantitative changes were still present after 2 and 4 weeks of recovery on air following 4 weeks of exposure to 1% CO₂.

DISCUSSION

During the first four weeks of exposure to 1% CO₂, the arterial Pco₂ level is elevated an

average of 4 mmHg. During this period the kidney fails to increase bicarbonate reabsorption, indicated by the slightly depressed standard bicarbonate. This is in marked contrast to the typical response of increased renal bicarbonate reabsorption that occurs during hypercapnia induced by higher CO₂ concentrations (Schwartz, Brackett, and Cohen 1965). We are therefore apparently dealing with a CO₂-induced metabolic acidosis. Metabolic acidosis is frequently a component during the early phase of respiratory acidosis in chronic hypercapnia induced by exposure to CO₂ concentrations between 3% and 15% CO₂, and it is during this phase that the most significant effects of CO₂ on various metabolic functions and morphological structure of the lung apparently occur (Schaefer 1974).

Four weeks of exposure to the slightly elevated arterial PCO₂ of 4 mmHg associated with a metabolic acidosis has been demonstrated to cause marked ultrastructural lung changes, which are still present after six weeks of exposure. More important, these alterations of the pneumocyte II cells persist even after a 4-week recovery period on air following four weeks of exposure to 1% CO₂. Since it is now generally accepted that type II alveolar pneumocytes are the cellular sites for biosynthesis of surface-active lecithins or pulmonary surfactant, one could speculate that prolonged CO₂ exposure increases rates of pulmonary surfactant production. Such a stimulation of type II pneumocytes has also been observed in the early phase of oxygen toxicity produced in rats by exposure to 70% O₂ (Redding, Arai, Douglas, Tsurutani, and Oven 1975) and 100% O₂ (Kistler, Caldwell, and Weibel 1967). An increase in tissue lecithin content paralleled the proliferation of type II cells (Redding et al. 1975). Measurements of lung lipids were not carried out in our studies.

The proliferation of type II pneumocytes during chronic exposure to 1% CO₂ suggests a compensatory reaction by type II pneumocytes to an impairing effect of the CO₂-induced acidosis on the alveolar lining cell (type I pneumocyte), since type II pneumocytes are considered the precursors of type I pneumocytes (Kistler et al. 1967). The question arises whether there is a common factor, e.g., acidosis, underlying the reported effects of exposure to increased CO₂ and O₂ on the ultrastructure of lungs. Unfortunately, blood gas measurements were not reported in the experiments of Redding et al. (1975) on the effects of exposure to 70% O₂, and therefore a comparison cannot be made with our experiment. The role of CO₂ in the etiology of lung damage caused by exposure to increased oxygen has been the subject of some debate in the literature and has not yet been determined (Wood 1975). Further experiments are required to answer this question. The findings of this study establish new criteria for the evaluation of low levels of chronic CO₂ toxicity that may occur in submarine and space craft environments.

The animals used in this study were handled in accordance with the provisions of Public Law 89-44 as amended by Public Law 91-579, the Animal Welfare Act of 1970 and the principles outlined in the Guide for the Care and Use of Laboratory Animals, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 78-23.—*Manuscript received for publication February 1977; revision received August 1977.*

Douglas, W. H. J., K. E. Schaefer, A. A. Messier, and S. M. Pasquale. 1979. Prolifération des pneumocytes II due à l'exposition prolongée à CO₂ (1%). *Undersea Biomed. Res. Sub. Suppl.*: S135-S142.—Des cobayes sont exposés à CO₂ (1%) dans un mélange de 21% O₂ et N₂; les animaux expérimentaux et les témoins (logés dans une chambre du même type et exposés à CO₂ atmosphérique) sont sacrifiés à intervalles d'une semaine. Une augmentation durable de PaCO₂ (4 mmHG en moyen) et une baisse légère de pH (−0,04 unités) sont observées chez les animaux exposés. Le taux de bicarbonate chez les témoins dépasse celui des expérimentaux de 1 ou 1,5 mEq, ce qui montre que le rein est incapable d'une réabsorption augmentée des bicarbonates. L'étude électro-microscopique des poumons met en évidence des modifications ultrastructurelles après 4

et 6 semaines d'exposition; le taux et la taille des cellules alvéolaires (pneumocytes II) sont augmentés. Ces changements sont observés encore 2 et 4 semaines après la fin de l'exposition, qui a duré 4 semaines. Ces modifications sont peut-être une réaction compensatoire aux effets de CO_2 sur la cellule alvéolaire (cellule du type I).

toxicité de CO_2
ultrastructure pulmonaire
acidose

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